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13. ABSTRACT ***(Maximum 200 Words)***

Osteoclast-mediated bone degradation is a debilitating result of metastasis to bone. During the metastatic tumor development, osteoclasts differentiate in the presence of high concentrations of transforming growth factor beta (TGF-ß). Our data support the hypothesis that TGF-ß is a survival factor for TGF-ß-induced osteoclasts. We had planned to test our hypothesis by pursuing the following two *objectives*: (1) Determine the effects of TGF-ß on tumor development in bone *in vivo* and (2) Determine the role of TGF-ß signal transduction in TGF-ß influences on mouse osteoclast-like cell survival. Objective 1 has proven difficult to achieve as the reagents that were planned for blocking activity *in vivo* were rapidly degraded in the animals. To circumvent this problem we generated cells that express either the dominant interfering or the constitutively active TGF-ß receptor. These have been injected in both the heart and bone to achieve the aims of this objective. The goal of objective 2 have been difficult to achieve the transfection technique induced apoptosis. We are currently establishing the adenoviral transfection technique in the laboratory to circumvent this difficulty. Overall, given the technological obstacles, we have made very good progress on the objectives of this proposal.

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FOREWORD

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INTRODUCTION

The purpose of this research is to determine the roles of transforming growth factor beta (TGF-ß) in metastatic tumor progression. The approach to this issue, both *in vivo* and *in vitro* studies are planned. In vivo studies will use two models in which TGF-ß activity will be blocked by employing both blocking antibodies and Latency Associated Protein: (1) direct injection of cells into the marrow cavity of nude mice and (2) employ cardiac injection. Direct injection will look at interactions within the bone compartment only whereas cardiac injection looks at survival in circulation, targeting to bone, and interactions within the bone itself. As detailed below, we had the initial obstacle that both the antibodies and LAP protein did not remain in circulation. As an alternative to these approaches, we developed MDA MB 231 breast cancer cell lines that express either the dominant interfering or constitutively active TGF-ß receptors. These have been cloned and confirmed to be expressing the appropriate protein and the state of the studies outlined below. The in vitro studies focus on the signaling pathway utilized by osteoclasts that differentiate in the presence of TGF-ß, when TGF-ß is a survival factor and cells differentiated without TGF-ß exposure, when addition of TGF-ß to the mature cells causes apoptosis. These studies are also progressing, as detailed below

BODY

Overall, as detailed below, we have made excellent progress on this study. Many of the tasks are underway. This report is detailed with specific reference to the Statement of Work.

Objective 1: Determine the effects of TGF-ß on tumor development in bone *in vivo*.

Task 1. Cardiac injections and antibody treatment: month 1 to month 16

We have discovered that the antibodies were too rapidly degraded and cleared for this approach to have much hope of working. Our alternate approach has been to create cell lines that constitutively express dominant active or inactive TGF-ß receptor constructs under the CMV promoter (these were gifts from Dr. Joan Massague). We have generated these cell lines from the MDA MB 231 cells under antibiotic selection. Initially, clones were screened for expression of the HA epitope tag. The appropriate response to TGF-ß treatment was used as a second screen in which the ability of TGF-ß to induce TGF-ß was examined. In these, the dominant active expressing cells expressed high levels of TGF-ß protein independent of TGF-ß treatment while the dominant interfering receptor expressing cells were not induced to express TGF-ß by TGF-ß treatment. These cells have been used for cardiac injection and direct bone injection to look at the role of TGF-ß in osteolysis. The cardia injection has been completed and the bones have been harvested. Thus we have completed this task.

Task 2: analysis of above: month 16 to month 30

As we have not yet reached month 16 of funding, we have not yet begun this part of the project. Bones have been harvested and imbedded. Sectioning is proceeding and should be completed within the next 6 to 9 months.

Task 3: Cardiac injections and LAP treatment : month 13 to month 24

Similar to the Task 1 difficulties, we have discovered that the LAP protein is rapidly degraded in vivo and we are substituting the alternate approach as outlined in Task 1.

Task 4: analysis of above: month 24 to month 36

See Task 2 above.

Task 5: Bone injections and antibody treatment: month 1to month 19

As noted in Task 1, antibodies were rapidly degraded in vivo. For this reason, we have generated the cells for both cardiac and bone injection as outlined above. The bone injection has been completed and the bones have been harvested. Thus we have completed this task.

Task 6: analysis of above: month 19 to month 32

As we have not yet reached month 19 of funding, we have not yet begun this part of the project. Bones have been harvested and are currently being imbedded. Sectioning will commence once the cardiac injected samples are sectioned.

Task 7: Bone injections and LAP treatment: month 15 to month 30

As with the antibodies, the LAP protein was rapidly degraded and we have used the cell lines as detailed in Task 5.

Task 8: analysis of above: month 30 to month 36

As we have not yet reached month 30 of funding, we have not yet begun this part of the project.

Objective 2: Determine the role of TGF-ß signal transduction in TGF-ß influences on mouse osteoclast-like cell survival

Task 9: receptor construct studies: month 1 to month 18

We have been working to perfect the transient expression studies. This has proven more difficult than we anticipated as the transfections techniques that have worked so successfully in avian osteoclasts induce apoptosis in murine osteoclasts. As we are examining an apoptotic response, this background is unacceptable. It has been recently documented that cells generated as we do can be successfully transfected to high efficiencies using adenoviral constructs. We are currently initiating the work to generated the necessary constructs in adenoviral vectors. As a first step, we are seeking University of Minnesota Safety approval of our facility for this work. Once we are approved, we plan to generate the appropriate vector constructs for these studies.

Task 10: SMAD pathway studies

interactions: month 13 to month 18: These studies have not yet begun as we are currently not in month 13 yet.

phosphorylations: month 18 to month 36: These studies have not yet begun as we are currently not in month 18 yet.

KEY RESEARCH ACCOMPLISHMENTS:

generation of cell lines from the MDA MB 231 cells that expresses a dominant interfering TGF-ß receptor construct and a separate cell line that expresses a dominant constitutively active TGF-ß receptor construct.

Successful cardiac injection of the above constructs.

Successful direct bone injection of the above constructs.

REPORTABLE OUTCOMES

2 abstracts to the 2001 American Society for Bone and Mineral Research annual meeting.

CONCLUSIONS:

We are making excellent progress on dissecting the roles of TGF-ß in tumor-driven osteolysis. We have successfully carried out both cardiac and bone injections of parental cells and cells expressing either a dominant interfering or a constitutively active TGF-ß receptor construct. In vitro studies have been hampered by the unexpected toxicity of the transfection technique. This is being dealt with by switching to adenoviral constructs for transfections.